

not change serum levels of cortisol binding globulin in patients with rheumatoid arthritis but it increases androstenedione relative to cortisol

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Background: Cortisol binding globulin (CBG) is produced by liver cells and is inhibited by proinflammatory cytokines such as interleukin (IL) 6. CBG serum levels are typically low during prolonged inflammatory processes. Thus, observed changes of cortisol during anti-tumour necrosis factor (TNF) treatment may be related to changes of CBG in rheumatoid arthritis (RA).

Objective: To investigate the course of CBG during anti-TNF treatment in RA.

Methods: 13 patients with longstanding RA, without prior prednisolone treatment, were included in this longitudinal study with subcutaneous adalimumab.

Results: Treatment with anti-TNF markedly decreased clinical markers of inflammation and serum IL6. Serum levels of cortisol, CBG, and the ratio of cortisol/CBG did not change markedly, whereas the ratio of serum CBG/IL6 increased ($p=0.004$). In parallel, levels of adrenocorticotrophic hormone decreased during the observation period. The ratio serum androstenedione/serum cortisol increased during the study ($p=0.036$).

Conclusions: During anti-TNF treatment relatively normal levels of CBG and a normal ratio of CBG/cortisol are found. Changes of cortisol in relation to IL6 during anti-TNF treatment, seen previously, may not be related to changes of CBG.

Table 1 Characteristics of the 13 female patients with RA

Characteristics	Patients with RA
Age (years)	58.2 (2.4) {48–73}
Disease duration (years)	6.5 (1.8) {0.5–20}
Baseline ESR (mm/ 1st h)	32.6 (5.5)
Baseline C reactive protein (mg/l)	143.4 (52.3)
Baseline serum IL6 (pg/ml)	23.2 (8.9)
Positive for rheumatoid factor, No (%)	13 (100)
Positive for antinuclear antibodies, No (%)	0 (0)
Baseline swollen joint score (points)	9.3 (0.6)
Baseline tender joint score (points)	10.9 (0.9)
<i>Additional treatment</i>	
Prednisolone, No (%)	0 (0)
Methotrexate, No (%)	13 (100)
Mean weekly methotrexate (mg)	8.7 (0.9)
NSAID (COX-1, COX-2), No (%)	12 (92)
COX-2 inhibitors, No (%)	5 (38)
Azathioprine, No (%)	0 (0)
Leflunomide, No (%)	0 (0)
Chloroquine/hydroxychloroquine, No (%)	0 (0)
Ciclosporin A, No (%)	0 (0)
Sulfasalazine, No (%)	0 (0)

Data are given as mean (SEM) {range} unless stated otherwise.

used a different anti-TNF agent adalimumab (to demonstrate possible class effects of anti-TNF antibodies).

PATIENTS AND METHODS

Patients and blood samples

In this study with adalimumab (Abbot SpA, Campoverde di Aprilia, Italy), we included 13 white patients with RA (all postmenopausal women) who fulfilled the American College of Rheumatology criteria for RA.¹⁵ The patients were selected according to the inclusion criteria of the Adalimumab Research in Active RA study (ReAct). None of the patients were receiving prednisolone or had received it previously (6 months before). All patients were given additional methotrexate (stable throughout this study) but no other immunosuppressive drugs (table 1). Patients were assigned to receive single self injections of adalimumab 40 mg subcutaneously every other week. Efficacy assessments included the American College of Rheumatology and EULAR response criteria (FA, PS-P).¹⁶ A baseline blood sample was taken 1–2 weeks before the start of the

Abbreviations: ACTH, adrenocorticotrophic hormone; ASD, androstenedione; CBG, cortisol binding globulin; HPA, hypothalamic-pituitary-adrenal; IL, interleukin; RA, rheumatoid arthritis; TNF, tumour necrosis factor

In a chronic inflammatory disease such as rheumatoid arthritis (RA), the hypothalamic-pituitary-adrenal (HPA) axis is altered: (a) patients have low spontaneous and stimulated cortisol secretion in relation to inflammation^{1–7}; (b) secretion of adrenocorticotrophic hormone (ACTH) relative to inflammation is inadequate⁷; and (c) adrenal androgens decrease.^{8–11} The causes of these alterations are only partly understood, but striking changes on all levels of the HPA axis seem to have a role. Cytokines such as interleukin (IL) 6 and tumour necrosis factor (TNF) are likely to play a prominent part in these alterations.

In a German RA cohort, we recently demonstrated that long term treatment with the anti-TNF agent infliximab sensitises the pituitary gland and favours adrenal androgen secretion.¹² Because production of cortisol binding globulin (CBG) may be inhibited by proinflammatory cytokines such as IL6,^{13 14} changes of cortisol, seen previously, may be dependent on changes of CBG serum levels. The present study in patients with RA receiving anti-TNF treatment aimed at investigating serum levels of CBG. We used a different cohort from Italy than in the earlier study¹² and

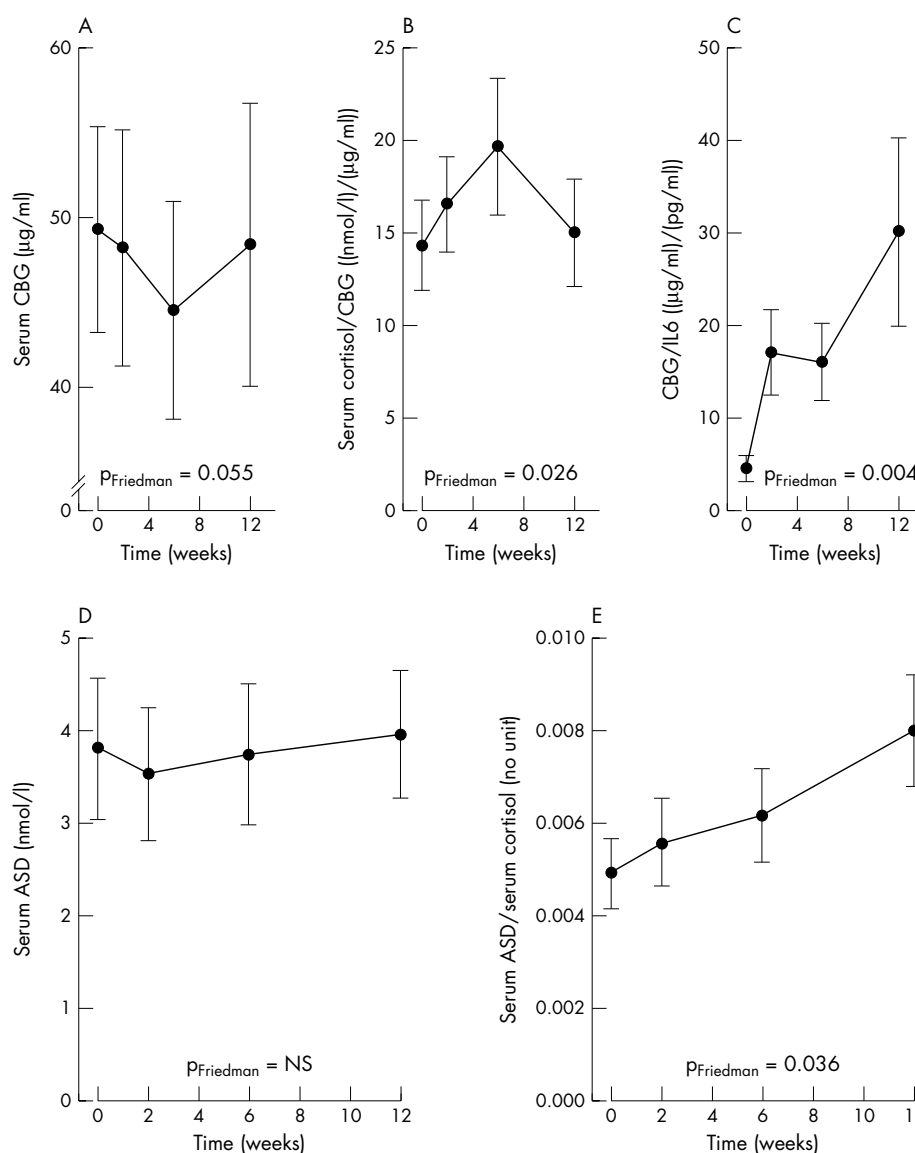


Figure 1 Course of serum cortisol binding globulin (CBG) and androstenedione (ASD) in relation to cortisol during 12 weeks of anti-TNF antibody treatment in patients with RA. Baseline values are given as time point 0. The graph depicts serum CBG (A), the ratio serum cortisol/CBG (B), the ratio serum CBG/IL6 (C), serum ASD (D), and the ratio serum ASD/cortisol (E). The data are given as means (SEM). The p value according to Friedman's test is given.

adalimumab treatment. Anti-TNF antibodies were infused on weeks 0, 2, 4, 6, 8, 10, and 12. For this study, patients were clinically investigated and blood was drawn between 8 00 and 9 00 am when the patients visited the outpatient clinic on the baseline day or at weeks 2, 6, and 12. The blood was immediately centrifuged and serum was stored at -80°C . The study was approved by the ethics committee of L Sacco University Hospital, Milan, Italy.

Laboratory variables

We used radioimmunoassays for the quantitative determination of serum levels of CBG (Biosource, Nivelles, Belgium) and cortisol (Coulter Immunotech, Marseilles, France). Serum levels of IL6 (high sensitivity Quantikine, R&D Systems, Minneapolis, MN, USA), androstenedione (ASD; IBL, Hamburg, Germany), and ACTH (Sangui BioTech, Inc, California, USA) were measured by immunometric enzyme immunoassays. Intra-assay and interassay coefficients of variation for all tests were $<10\%$.

Statistical analysis

Means between different time points during the course of anti-TNF treatment were compared by Wilcoxon's signed rank test for paired data (SPSS/PC, Advanced Statistics, version 11.5.1, SPSS Inc, Chicago). A decrease or increase of a variable over time (during anti-TNF treatment) was tested by the non-parametric Friedman test (SPSS). A value of $p < 0.05$ was the significance level.

RESULTS

Anti-TNF treatment with adalimumab decreased the swollen joint count (baseline v 12 weeks: $9.3 (0.6) v 2.9 (0.6)$, $p = 0.001$), tender joint count ($10.9 (0.9) v 6.5 (0.4)$, $p = 0.005$), and serum levels of IL6 ($23.2 (8.9) v 3.2 (1.0)$, $p = 0.002$).

Because cortisol determination may be influenced by presence of serum CBG, we measured this particular transport protein in this study. Baseline serum CBG was normal, and serum CBG did not markedly change during the

treatment (fig 1A). Serum cortisol in relation to serum CBG also did not change during anti-TNF treatment (fig 1B). The ratio of serum CBG/IL6 significantly increased during the observation period (fig 1C).

ASD is one of the major adrenal androgens. The ratio of serum ASD/cortisol increased during the observation period, which indicates that adrenal androgen secretion increases relative to cortisol secretion (fig 1E).

In addition, levels of ACTH decreased during the 12 weeks of anti-TNF treatment (baseline ν 12 weeks: 1.6 (0.1) ν 1.2 (0.1) pmol/l, $p=0.007$), but cortisol remained relatively stable (615.3 (81.5) ν 569.6 (77.1) nmol/l). We recently demonstrated that levels of hormones in relation to serum IL6 can be used to estimate HPA axis activity in relation to this cytokine.⁷ This technique demonstrated that ACTH and cortisol serum levels increased relative to serum levels of IL6 (ACTH/IL6: 0.46 (0.28) ν 0.86 (0.24) (pmol/l)/(pg/ml); cortisol/IL6: 62.1 (22.8) ν 390.2 (108.1) (nmol/l)/(pg/ml)). This indicates that the main hormones of the HPA axis normalise relative to serum IL6. For ACTH and cortisol, serum levels are respectively two and four times higher in relation to IL6 at 12 weeks than at baseline.

DISCUSSION

CBG binds glucocorticoid hormones and regulates their biological availability to target cells.¹⁷ CBG is mainly produced by the liver in all species examined.¹⁸ IL6 and other proinflammatory cytokines can decrease production of CBG from liver cells.^{13–14} Thus, the proinflammatory load may decrease CBG serum concentrations in RA, which has been demonstrated in patients with prolonged critical illness in intensive care units.¹⁹ In this study, CBG baseline levels were normal in patients with RA. Furthermore, CBG serum levels did not markedly change during the course of anti-TNF treatment. In view of these findings, we suggest that measurement of serum levels of cortisol are not markedly influenced by CBG concentrations in patients with RA.

In addition to the marked improvement of clinical and laboratory measures of inflammation, treatment with adalimumab decreased IL6 secretion. IL6 can be considered as a hormone which informs the adrenal gland and the central nervous system about the inflammatory status of peripheral joints.²⁰ It is important to mention that the hypothalamic-pituitary axis, particularly, undergoes desensitisation upon repetitive IL6 stimuli, with a marked unresponsiveness of ACTH secretion.^{21–22} Removal of the IL6 proinflammatory load by anti-TNF treatment leads to increased cortisol levels relative to IL6. This phenomenon is probably not much influenced by CBG because this transport protein remains constant. This study also demonstrated that ACTH serum levels decrease during anti-TNF treatment, which also shows that the proinflammatory load stimulates ACTH secretion. The improvement of the hypothalamic-pituitary function can further be demonstrated when serum levels of ACTH are expressed relative to IL6.

Earlier studies have shown that adrenal androgen secretion is low in patients with RA.^{8–11} In our earlier study we demonstrated relatively normal serum levels of ASD during the course of anti-TNF treatment.¹² This was confirmed in this present study in Italian patients with RA treated with a different anti-TNF antibody. The relative preponderance of the glucocorticoid pathway (to cortisol) in relation to the androgen pathway (to ASD) was seen when the ratio of serum cortisol/ASD was used. This particular ratio increased during anti-TNF treatment with infliximab,¹² which was confirmed in this present study with adalimumab. These findings suggest that anti-TNF treatment favours ASD secretion relative to cortisol.

In conclusion, our present anti-TNF study with adalimumab in an Italian RA cohort demonstrated that CBG levels are not markedly altered during anti-TNF treatment. Thus, measurement of cortisol levels during anti-TNF treatment relative to serum levels of IL6 is probably not influenced by CBG levels. Furthermore, we confirmed some of our recent findings in a German RA population using infliximab. Both studies showed that the HPA axis relative to IL6 was normalised and that adrenal androgens were increased relative to cortisol. These positive effects must be considered as an additional systemic anti-inflammatory influence of anti-TNF treatment in patients with RA.

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